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Stock Structure Analysis of Channidae Family from River Sutlej in Punjab by using Truss Networking System

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Abstract: The present study was carried out to distinguish the phenotypic stocks of fish species under Channidae family from selected stretches of river Sutlej in Punjab based on morphometric variations using truss network system. Sampling was conducted at monthly intervals from three different sampling sites of Sutlej river i.e. Rupnagar, Ludhiana and Harike pattan from November 2020 to October 2021. A total of 120 specimen representing different age groups of three fish species under Channidae family (*Channa striata, C. marulius* and *C. punctata*) were analysed. Truss network was constructed by interconnecting 12 homogenous landmarks form 26 truss distances. *tpsUtil32, tpsdig2* software packages were used to extract truss distances from digital photos of specimens. Under principal component analysis (PCA); PC1, PC2 and PC3 accounted 92.9, 3.5 and 1.2% variance in *C. striata*, whereas 86.3, 7.2 and 6.5% variance was observed in *C. marulius. C. punctata* accounted 44.8, 35.4 and 17.7% variance in PC1, PC2 and PC3, depicting highest and lowest variability in *C. striata* and in *C. marulius*, respectively. The study revealed no significant morphometric differences within species, indicating the presence of same phenotypic stock of *Channa* species in River Sutlej. The outcome of the study can be a scientific base for future selective breeding and river ranching programme.

Keywords: River Sutlej, Channidae, Phenotypic characters, Truss networking system, Principle component analysis

River Sutlej is one of most important tributaries of Indus river system. It is the longest tributaries with total length of 1450 km, out of which 740 km lies in India. It originates from trans-Himalayas at an elevation of 4630 msl at south-west of Tibetian lakes, Rakshastal. Shipki pass of Great Himalayan range (Himachal Pradesh) is a place where river Sutlej enters into India. It enters the plains near district Rupnagar in Punjab and flows up to Fazilka via industrial city of Ludhiana after confluence with river Beas at Harike Pattan. In the realm of ecosystem-based fisheries management, fish stock characterization is considered as crucial because stocks with a variety of life cycle characteristics, such as growth, mortality and reproductive potential, are thought to be vital for fisheries management. Different body shapes within a species result from difference in environmental factors, genetic makeup are frequently observed (Cadrin 2000). Differences in growth and maturation rates are the indications of patterns of morphometric variation in fish which, in turn, are the result of ontogeny. One of the most common and cost-effective approaches for stock identification is biometric features. To overcome the inherent flaws of standard morphometric methods, the 'Truss network system,' a system of morphometric measurements that effectively discriminates phenotypic stock structure and population features, has become increasingly popular for stock identification (Cadrin 2005). It is more accurate and modern technique of classification of individuals than traditional morphometric methods since it helps to overcome size dependent variance and takes shape-related characteristics into account (Strauss and Bookstein 1982). Dean et al (2001) observed that truss analysis distinguishes physically identical species. Zhang et al (2016) mentioned that the truss network could be attributable to spatial variation in the environmental factors of different locations. Many researchers employed truss morphometry to distinguish between fish stocks of Indian waters such as *Catla*, *Labeo rohita, Rastrelliger kanagurta* and *Megalaspis cordyla* (Jayasankar et al 2004, Sajina et al 2011, Ujjania and Kohli 2011) and also worked on *Channa* species like *C. punctatus*, *C. striata* and *C. gachua* (Khan et al 2013, Kashyap et al 2016, Jearranaiprepame et al 2017, Khan et al 2019).

There are three genera (*Aenigmachanna, Channa* and *Parachanna*) and 50 species available in the Channidae family (Actinopteri, Anabantiformes) (Britz et al 2019). The extant three species of *Parachanna* are African, despite *Aenigmachanna and Channa* that two genera are purely Asian. Britz et al (2019) also reported a new species from Malappuram, Kerala i.e. *Aenigmachanna gollum*. Due to the appearance of massive scales on the skulls of most species, Channids are popularly referred to as "Snakeheads". The fish species under Channidae family have huge demand in domestic and international markets because of their taste, absence of intramuscular spine, flavour and medicinal values. In Indian waters phylogenetic study using truss networking system was conducted on *C. punctata* (Khan et al 2013) and *C. striata* (Kashyap et al 2016) but no related study has been conducted on *C. marulius*. Therefore, the current study was designed to use truss network system, a modern morphometric method, to separate the phenotypic stocks of Channidae species collected from selected stretches of river Sutlej in Punjab to determine morphometric variations.

MATERIAL AND METHODS

Sample collection: The study was conducted at the College of Fisheries, GADVASU, Ludhiana, Punjab for a duration of November 2020 to October 2021. Sampling was conducted at monthly intervals from three different sampling sites of Sutlej river i.e.Rupnagar Head works (30°59′ 52.9404″N, 76°32′ 00.636″E), Rail/road Bridge at Phillaur, Ludhiana (30°59′ 35.2608″N, 75°47′ 28.2516″E) and Harike Pattan (31°08′ 32.334″N, 74°56′ 55.0032″E) with a distance of about 90 km from one station to another. A total of 120 specimens out of which *Channa striata* (n=40), *C. marulius* (n=40) and *C. punctata* (n=40) of different age groups were collected from selected sites.

Sample preparation and measurement: Measurements of fish samples were taken by piercing the paper with a needle at relevant anatomical landmarks. Fish samples were placed on a water-resistant laminated graph sheet. To identify each specimen in the graph, it was labelled with a unique code. Freshly caught samples were washed properly under running water, drained and wiped dry before taking images. The digital image of each individual was obtained using a Sony (Japan) camera at a fixed distance (Zhang et al 2016). Digital images of fish specimens implied a complete body shape also allow making repeated measurements (Cadrin and Friedland 1999). Images were captured from the same height as well as angle to minimize the human error as per procedure described by Mir et al (2013).

Statistical analysis: The truss network was constructed by interconnecting the 12 homogenous landmarks form 26 truss distances (Table 1). The network extended across the whole body of the fish to depict the full dimension of the body (Moore and Bronte 2001 and Mir et al 2013).

Measurement of truss distances: *tpsUtil32, tpsdig2* (Rohlf 2006) and the PAST software package (Hammer et al 2001) were used to extract truss distance from digital photos of specimens. The *tpsUtil32* software converted the 'JPEG/JPG' format to 'TPS' file as *tpsdig2* software works only in TPS file. The *tpsdig2* software set the landmarks and measured the truss distances of each captured image (Fig. 1). All the calculated measurements were transferred to Paleontological Statistics (PAST) software package in a spread sheet file (Hammer et al 2001) and the coordinate data (X-Y) was transformed into linear distances using the Pythagorean Theorem by the software for subsequent analyses.

Normalization of the data: For the analysis of morphometric data, an important stage in the data preparation was to eliminate the size effect while comparing the different sizes of the fish. Considering allometric growth, before statistical analyses all the measurements were log transferred to remove the size effect by applying an allometric approach. The significant correlation exists between the body length and the morphometric variables and hence, the variation in

Table 1. Particulars of truss distances between landmarks used in fish species under Channidae family

Landmark numbers	Truss distance
1-2	Tip of snout- Termination point of snout
1-12	Tip of snout- Termination of mouth
2-12	Termination point of snout - Termination of mouth
2-3	Termination point of snout – Forehead
11-12	Most posterior point of maxillary- Termination of mouth
2-11	Termination point of snout - Most posterior point of maxillary
3-12	Forehead - Termination of mouth
3-11	Forehead - Most posterior point of maxillary
3-4	Forehead - Origin of dorsal fin
9-10	Origin of Anal fin- Point of Pectoral fin insertion
10-11	Point of Pectoral fin insertion- Most posterior point of maxillary
3-9	Forehead- Origin of Anal fin
3-10	Forehead- Point of Pectoral fin insertion
4-11	Origin of Dorsal fin- Most posterior point of maxillary
4-10	Origin of Dorsal fin- Point of Pectoral fin insertion
4-5	Origin of Dorsal fin- Termination of dorsal fin
8-9	Termination of Anal fin- Origin of Anal fin
4-8	Origin of dorsal fin- Termination of Anal fin
5-9	Termination of Dorsal fin- Origin of Anal fin
4-9	Origin of dorsal fin- Origin of Anal fin
5-6	Termination of dorsal fin- Dorsal side of Caudal fin
7-8	Ventral side of Caudal fin- Termination of Anal fin
5-7	Termination of Dorsal fin- Ventral side of Caudal fin
6-8	Dorsal side of Caudal fin- Termination of Anal fin
5-8	Termination of Dorsal fin -Termination of Anal fin
6-7	Dorsal side of Caudal fin- Ventral side of Caudal fin

the whole data may discriminate the populations based on the size of fish. Therefore, each distance was corrected as per Elliott et al (1995).

$$M_{adj} = M\,(L_s / L_0)^{b}$$

Where, M_{adj} = Transformed the Truss measurement, M = Original Truss measurement

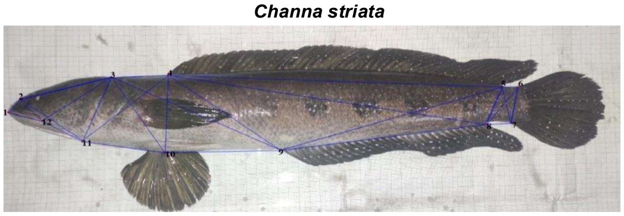
Ls = Overall mean standard length, L_o = Standard length of Fish

'b' is the within group slop regression for plot of M and L_s on logarithmic scale

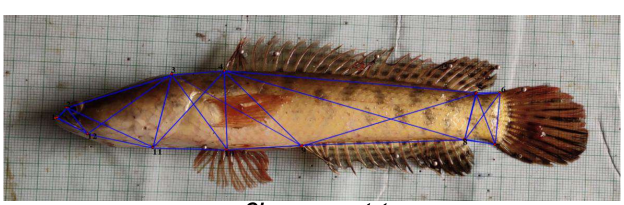
Since variation should be immutable to body shape thus it should not be related to the relative size of fish (Reist 1985). The significance of the connection between transformed variables and standard length was tested on the standardized data (Turan 1999).

The Principal component analysis was performed to understand that which of the morphometric measurements could distinguish populations. The PCA was based on correlation or covariance matrix which considered all the data simultaneously rather than individually (Bookstein et al 1985). Eigen values were obtained using this procedure, which allowed for the major part of the variation of original variables to be explained with a small number of factors. The proximity in the space defined by the components is used for analyzing the relationship between populations (Khan et al 2012). PCA was performed using PAST software.

Channa striata



Channa marulius



Channa punctata

1. Tip of Snout 2. Termination point of Snout 3. Forehead 4.Origin of Dorsal fin 5. Termination of Dorsal Fin 6. Dorsal side of Caudal fin 7. Ventral side of Caudal fin 8. Termination of Anal fin 9. Origin of Anal fin 10. Point of Pectoral fin insertion 11. Most posterior point of Maxillary 12. Termination of Mouth

Fig 1. Consensus of Truss Morphometric Network (TMN) of *C. striata, C. marulius, C. striata* and *C. punctata*

RESULTS AND DISCUSSION

The three species under family Channidae were recorded i.e. *C. striata, C. marulius* and *C. punctata* from the selected stretches of River Sutlej in Punjab. Among these *C. marulius* was dominant in overall catch and available in all the three sites and seasons whereas, *C. striata* and *C. punctata* were at Site 2 (Ludhiana) and Site 3 (Harike) and it was absent at Site 1 (Rupnagar). *C. marulius* was dominant fish in catch throughout the study period (weight basis) under family Channidae (58%) followed by *C. striata* (37%) and *C. punctata* (5%) contributed significantly. In *C. striata* average weight (W), total length (TL) and standard length (SL) were recorded as 570.58 g, 41 cm, 34.97 cm, respectively. In *C. marulius* W, TL and SL were as 1482.39 g , 57.70 cm and 49.94 cm respectively. The W, TL and SL were recorded as 37.43 g , 16.88 cm, 14.10 cm respectively in *C. punctata*. Output data of the truss networking system was further analyzed through PCA to find out the interrelationship among intra and inter species variability. The first component (PC1) of morphometric is interpreted as the size axis (Bookstein et al 1985) and the second (PC2) and third components (PC3) as shape variables (Humphries et al 1981, Bookstein 1989, Sundberg 1989). In present study the main two factors which were responsible for the association among the truss measures were shape and size of the fish. The correlation coefficient between standard length and truss measurements were closed to one before the transformation for size correction. After the transformation, truss measurements did not show significant correlation with standard length of the fish species and were ready for further analysis. PC1, PC2 and PC3 of *C. striata* successively accounted 92.9, 3.5 and 1.2% variability, respectively. PC1, PC2 and PC3 contributed 86.3, 7.2 and 6.5%, of total variance in *C. marulius* whereas PC1, PC2 and PC3 contributed 44.8, 35.4 and 17.7% variability in *C. punctate* (Table 2 and Fig. 2-4).

In all of the Channidae species studied, the overall findings through PCA revealed that size is the most important contributor to variability, followed by shape. Khan et al (2013) studied the PCA of *C. punctata* (n=234) from three different River of India (i.e Ganga, Yamuna and Gomati) and find the different stock of *C. punctata*. Kashyap et al (2016) reported 83.25, 5.33 and 3.37% variability in PCI, PCII and PCIII, respectively in *C. punctata* from Northern and Eastern Regions of Gomati River in India. Jearranaiprepame (2017) calculated the PCA of population of one of the species *C. gachua* under same family Channidae; and observed that first three components were contributing 49.98% variability due to body shape. Norainy et al (2018) analysed size and shape variation of four *Channa* species (*Channa striata, C. micropeltes, C. marulioides and C. lucius*) through truss networking system from Indonesian waters. First three components of PCA contributed 88.98% variability due to body size and 46.05 % variability due to body shape. Khan et al (2019) examined the PCA of *C. striata* population from the river Ganga as well as from its tributaries Yamuna and Gomti and found that the first three components were responsible for 40.2, 9.86 and 6.68% of variability due to body size.

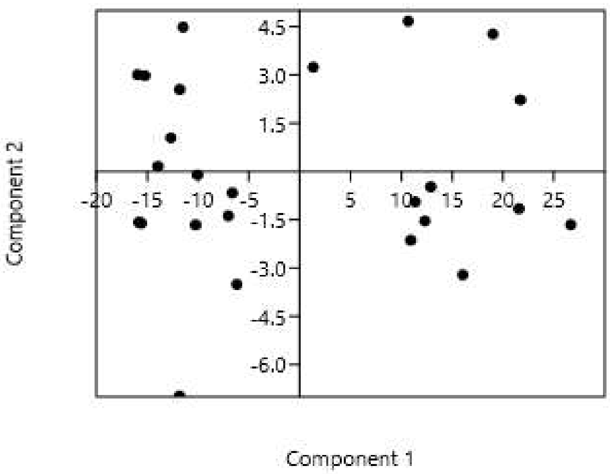


Fig. 2. Principal component analysis of *C. striata* (intra species) collected from river Sutlej

In present study, first five components, the eigenvalues of *C. striata* along river Sutlej was higher than one (209.5, 8.1, 2.7, 1.6 and 1.2, respectively). Higher eigenvalue factors contributed more to the variations in the variables, while lower eigenvalue factors have been dismissed as redundant with more relevant factors. The eigenvalue of the first six in *C. marulius* were also higher than one (98.8, 8.2, 7.5, 5.7, 4.5 and 1.8, respectively). In *C. punctata*, the eigenvalue were recorded more than one for the first five components (57.7, 45.6, 22.8, 2.5 and 1.0, respectively). Fig 2 - 4 depicted the intra species scatter plots of the *C. marulius, C. striata* and *C. punctata*. Based on the morphometric characterization

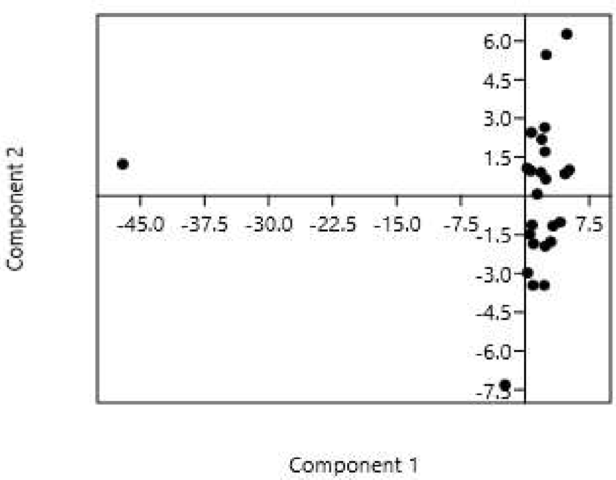


Fig. 3. Principal component analysis of *C. marulius* (intra species) collected from river Sutlej

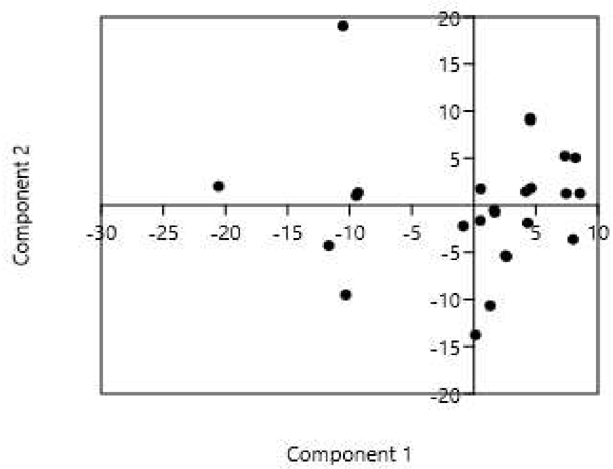


Fig. 4. Principal component analysis of *C. punctata* (intra species) collected from river Sutlej

Table 2. Percentage of variance accounted by variables having Eigen values more than 1 of within group PCA for *Channa striata, Channa marulius* and *Channa punctata* from Sutlej River

Principal component	*Channa striata*		*Channa marulius*		*Channa punctata*	
	Eigenvalue	% Variance	Eigenvalue	% Variance	Eigenvalue	% Variance
1	209.533	92.91	98.830	86.317	57.7525	44.812
2	8.113	3.597	8.2843	7.2354	45.6752	35.441
3	2.798	1.240	7.536	6.582	22.8739	17.748
4	1.699	0.753	5.718	4.994	2.517	1.953
5	1.212	0.537	4.5627	3.9851	1.097	0.851
6			1.818	1.588		

maximum variability within stock was observed in *C. striata* and it was minimal in *C. marulius*. No significant morphometric differences were recorded among three species. Khan et al (2013) studied the PCA of *C. punctata* (n=234) from three different River of India (i.e Ganga, Yamuna and Gomati) and find the different stock of *C. punctata*. Kashyap et al (2016) reported 83.25, 5.33 and 3.37% variability in PCI, PCII and PCIII, respectively in *C. punctata* from Northern and Eastern Regions of Gomati River in India. Jearranaiprepame (2017) calculated the PCA of population of one of the species *C. gachua* under same family Channidae; and observed that first three components were contributing 49.98% variability due to body shape. Norainy et al (2018) analysed size and shape variation of four *Channa* species (*Channa striata, C. micropeltes, C. marulioides and C. lucius*) through truss networking system from Indonesian waters. First three components of PCA contributed 88.98% variability due to body size and 46.05 % variability due to body shape. Khan et al (2019) examined the PCA of *C. striata* population from the river Ganga as well as from its tributaries Yamuna and Gomti and found that the first three components were responsible for 40.2, 9.86 and 6.68% of variability due to body size.

CONCLUSIONS

In Channidae family *C. marulius* was dominant fish in catch composition followed by *C. striata* and *C. punctata* contributed significantly. The truss analysis revealed no significant morphometric differences, indicating the presence of same phenotypic stock of *Channa* species in River Sullej. Among different species under Channidae family maximum variability was observed in *C. striata* and least in *C. marulius.*

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